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Metagenomics of Bolidophyceae in Plankton and Ice of the White Sea

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Abstract—The molecular diversity of poorly studied algae of Bolidophyceae class was first estimated by Illumina sequencing of V4 region of 18S rRNA gene in ice, under-ice water and summer water of the subarctic White Sea. We used two clustering thresholds – 93 and 97% – and revealed 31 phylotypes of Bolidophyceae. *Triparma pacifica* and *T. strigata* were identified to species level. The association of individual phylotypes to certain biotopes (ice or plankton) and stages of seasonal succession (under ice or summer plankton) has been established. Some phylotypes are found in different biotopes and over a wide temperature range. Due to changing their genetic composition, Bolidophyceae are a constant component of the photoautotrophic plankton and ice communities.

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The class Bolidophyceae was described less than 20 years ago and included, at the time of description, only the genus *Bolidomonas* with two species of flagellated marine algae: *B. pacifica* and *B. mediterranea* [1]. The class Bolidophyceae is assigned to Heterokonta because *Bolidomonas* cells have two flagella of unequal length and some peculiarities of cell structure typical of representatives of this group. *Bolidomonas* cells are no more than 2 μm in size, and therefore representatives of this genus correspond to the pico-size fraction (<3 μm) of plankton and ice flora.

Phylogenetic analysis using nuclear and chloroplast genes has revealed a close relationship between Bolidophyceae and diatoms [1, 2], and Bolidophyceae is considered as an intermediate group between diatoms and other heterokont algae [1]. The subsequent phylogenetic studies extended the class Bolidophyceae due to the inclusion of the order Parmales [3], previously classified as golden-brown algae (class Chrysophyceae).

Originally, the order Parmales included nonflagellated (non-motile) marine algae, the cells of which are covered by silicified plates (from five to nine plates) [4]. The sizes of most of the Parmales correspond to the picofraction. The peculiarities of plate morphology, revealed only with a scanning electron microscope (SEM), made it possible to describe the following three genera within the order Parmales: *Pentalamina* (one species), *Tetraparma* (four species), and *Triparma* (five species, four subspecies, four forms). Phylogenetic analysis with nuclear, chloroplast and mitochondrial genes was a basis for assigning the previously described motile naked *Bolidomonas* to the genus *Triparma* (*T. pacifica* [1, 5] and *T. mediterranea* [5]). The life cycle of at least some species of the genus *Triparma* may include a motile naked form and non-motile silicified form [5].

It is very difficult to determine the composition of Bolidophyceae in natural communities by microscopic methods because of their small cell size. For example, SEM is required to identify the silicified Bolidophyceae. The motion pattern should also be taken into consideration for reliable identification of flagellated forms, which requires examination of live samples and/or culturing. Molecular approach offers extensive possibilities for assessing the composition of the smallest photoautotrophs, including Bolidophyceae, in natural communities. The metagenomic analysis of the small-cell (<5 μm)

Abbreviations: BA, Bayesian analysis; ML, maximum likelihood (method); OTU, operational taxonomic unit; st., station.

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